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Vitamin C in the human stomach: relation to gastric pH, gastroduodenal disease, and possible sources

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SUMMARY **Fasting gastric juice pH and concentrations of vitamin C in gastric aspirate and plasma were measured in 73 patients undergoing endoscopy. Vitamin C concentrations were significantly lower in those with hypochlorhydria (pH>4; n=23) compared with those with pH≤4 ($p<0.005$) and there was a significant correlation between gastric juice and plasma concentrations ($p=0.002$). Patients with normal endoscopic findings had significantly higher intragastric concentrations of vitamin C than those with gastric cancer ($p<0.001$), pernicious anaemia ($p<0.005$), gastric ulcer ($p<0.01$), duodenal ulcer ($p<0.05$), or after gastric surgery ($p<0.01$). There was a strong trend ($0.05<p<0.1$) towards lower intragastric concentrations of vitamin C in patients with chronic atrophic gastritis. *In vitro*, vitamin C concentrations remained stable in acidic but fell significantly over 24 hours in alkaline gastric aspirate. Gastric secretory studies in five volunteers showed that vitamin C concentrations increased significantly after intramuscular pentagastrin. These findings suggest that the low fasting levels of vitamin C in hypochlorhydric gastric juice may be caused by chemical instability and that vitamin C may be secreted by the human stomach.**

Epidemiological studies[1-3] show an inverse relationship between gastric cancer risk and consumption of foods rich in vitamin C. Moreover, the increased intake of such foods in preference to smoked and cured produce has been postulated as one of the factors accounting for the decline in gastric cancer mortality over the past few decades in most countries where reliable statistics are available.[4,5]

Gastric cancer may be caused by exposure of the mucosa to N-nitroso compounds[1] which are formed in the stomach by the interaction of nitrite and nitrosatable substrates. Vitamin C is an effective scavenger of nitrite, reducing it to nitric oxide and preventing nitrosamine formation *in vitro*[6] and *in vivo*.[7] Furthermore, oral supplements of vitamin C can significantly reduce mutagenic activity in human gastric juice.[8] Despite the evidence of a possible protective role, little attention has been paid to vitamin C concentrations in gastric juice. Previously we have shown that pretreatment with vitamin C increases basal fasting concentrations of vitamin C in the stomach.[8] The aim of this study was to assess the relationship between basal intragastric concentrations of vitamin C and gastric pH, gastroduodenal disease, gastric mucosal pathology and plasma levels. The possible sources of vitamin C in the stomach were also investigated.

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Table *Details of patients studied*

Group	n	Age (yr) Mean	Age (yr) Range	Sex M:F	Length of follow-up after operation (yr) Mean	Length of follow-up after operation (yr) Range
Normal endoscopy	13	37·9	23–58	6:7		
Duodenal ulcer	18	50·6	23–76	13:5		
Gastric ulcer	13	58·3	40–72	7:6		
Gastric cancer	12	66·7	60–77	10:2		
Pernicious anaemia	4	63·0	58–70	1:3		
Postoperative*	13	62·0	34–82	12:1	17·2	1–40

*3 Billroth I partial gastrectomy; 5 Billroth II partial gastrectomy; 3 Truncal vagotomy and gastroenterostomy; 2 Truncal vagotomy and pyloroplasty.

Methods

PATIENTS

The 73 patients studied were divided into six groups (Table). All underwent oesophagogastroduodenoscopy after an overnight fast as part of routine investigations and gave written consent to the procedure. None was receiving H_2 receptor antagonists at the time of the study. Pernicious anaemia was confirmed by the presence of megaloblastic anaemia, a low serum $-B_{12}$ level, a diagnostic Schilling test and response to treatment with vitamin B_{12}.

SAMPLING TECHNIQUES

Immediately before endoscopy a sample of venous blood (3–5 ml) was taken from 54 of the 73 patients (74%) for measurement of plasma vitamin C including all patients in the pernicious anaemia and postoperative groups, nine of 13 in the normal endoscopy group (62%), 14 of 18 duodenal ulcer patients (78%), 12 of 13 gastric ulcer patients (92%), and two of 12 patients with gastric cancer (17%). At endoscopy, a sterile Teflon cannula was passed through the biopsy channel of the endoscope and 5–10 ml of gastric contents were aspirated, taking care to avoid trauma to the mucosa and contamination with blood.

After aspiration, 52 patients (71%) had at least two gastric biopsy specimens taken from uninvolved mucosa in the area within 5 cm of the pylorus, and within 5 cm of the stoma in the resected group including all patients in the gastric ulcer and pernicious anaemia groups, six of 13 in the normal endoscopy group (46%), 10 of the 18 duodenal ulcer patients (56%), eight of the 12 patients with gastric cancer (75%), and 11 of the 13 patients in the postoperative group (85%). Additional specimens were taken from any pathological lesions seen.

A total of 42 patients (58%) had gastric and plasma vitamin C measured and gastric biopsy specimens taken.

MEASUREMENT OF VITAMIN C

Immediately after aspiration a 1 ml aliquot of gastric juice was mixed with 2 ml 5% trichloroacetic acid to precipitate protein which was then removed by centrifugation. Approximately 50 mg of activated charcoal was added to the supernatant and agitated for 15 minutes to oxidize ascorbic to dehydroascorbic acid and clarify the solution. This was followed by positive pressure filtration through a Millipore filter (pore size 8 μm). The resulting extract of gastric juice was then analysed spectrophotometrically using Lowry's modification[9] of the 2,4-dinitrophenylhydrazine method described by Roe and Kuether.[10] Plasma vitamin C concentrations were also measured using this technique as originally described.[10]

This technique measures total vitamin C, that is ascorbic acid, dehydroascorbic acid, and diketogluconic acid (the unstable breakdown product of dehydroascorbic acid). In man, separate measurements of these components in plasma suggest that about 80–90% of the measured material is in the form of ascorbic acid.[11]

Vitamin C concentrations were measured without knowledge of the endoscopic findings, gastric pH and other patient data.

MEASUREMENT OF GASTRIC pH

The pH of the gastric aspirate was measured with a combined glass electrode calibrated at pH 4 and 7. Hypochlorhydria was defined as a fasting gastric pH>4.

HISTOLOGICAL ASSESSMENT OF GASTRIC MUCOSAL BIOPSY SPECIMENS

The biopsy specimens were orientated on filter paper and immediately fixed in formol saline. Paraffin processed sections were cut at three levels and stained with haematoxylin and eosin. The sections were examined 'blind' and specifically assessed for the presence of chronic gastritis using Whitehead's classification.[12]

DAILY INTAKE OF VITAMIN C

Daily intake of vitamin C was estimated using a dietary recall questionnaire. Patients were asked to give details of the frequency of ingestion of a small number of food items including green vegetables, oranges, fruit juices, and potatoes, which account for most of the vitamin C intake in man.

STABILITY OF VITAMIN C IN GASTRIC JUICE

Experiments were performed *in vitro* to assess the stability of vitamin C in gastric juice. Two samples of gastric juice, one acidic (pH 1·2, obtained from a duodenal ulcer patient) and one alkaline (pH 7·2, from a Billroth II partial gastrectomy patient) were each divided to provide two 10 ml subsamples. One subsample acted as a basal aliquot and the other was supplemented with vitamin C to increase the concentration of the vitamin by 4 mg/dl. The four subsamples were then incubated in a 37°C water bath for 24 hours and 1 ml aliquots taken for measurement of vitamin C and pH at 0, 1, 2, 4, 8, and 24 hours.

GASTRIC SECRETORY STUDIES

The possibility of secretion of vitamin C by the gastric mucosa was investigated using the conventional 'short' pentagastrin test in five volunteers (two men, three women; mean age 51 years, range 35–70) without any clinical evidence of gastrointestinal disease. After collecting a basal one hour sample,

pentagastrin (6 μg/kg body weight) was given by intramuscular injection and secretions were collected in 10 min aliquots for 40 min. One ml of the basal and four postpentagastrin samples was taken for vitamin C analysis. The pH of each sample was measured and the acid output determined by titration.

STATISTICAL ANALYSIS

Differences between the groups were analysed by the Wilcoxon's rank-sum test for statistical significance. The relations between intragastric and plasma vitamin C concentrations and between these concentrations and estimated daily intake of vitamin C were analysed by Kendall's rank correlation test. A p value less than 0·05 was considered significant.

Results

VITAMIN C AND GASTRIC pH

Of the 73 patients studied, 23 (32%) were hypochlorhydric (pH>4) whilst 50 had a gastric pH≤4. Intragastric concentrations of vitamin C were significantly lower in hypochlorhydric patients compared with those pH≤4 ($p<0·005$) (Fig. 1). Of the 54 patients who had plasma vitamin C measured, 18 (33%) were hypochlorhydric and 36 had a gastric pH≤4. Plasma concentrations were also significantly lower in hypochlorhydric subjects ($p<0·005$) (Fig. 2).

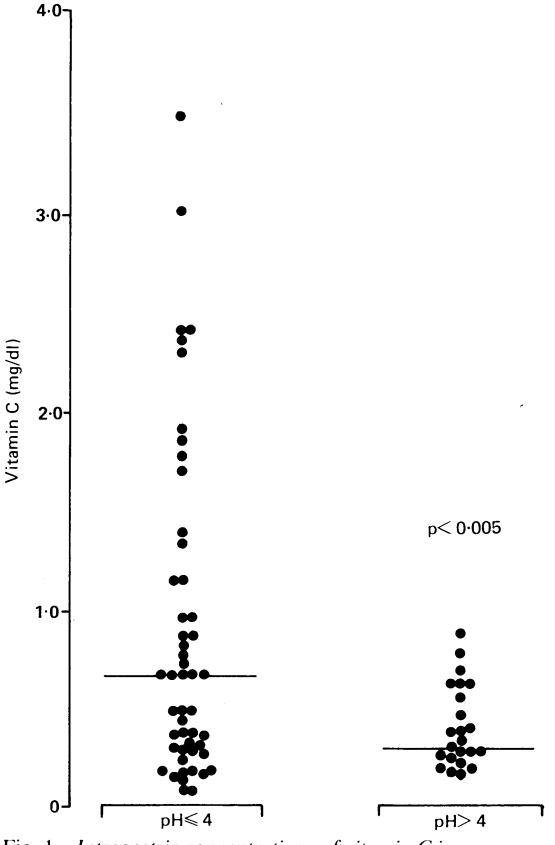

Fig. 1 *Intragastric concentrations of vitamin C in hypochlorhydric patients compared with patients pH≤4. ——=median.*

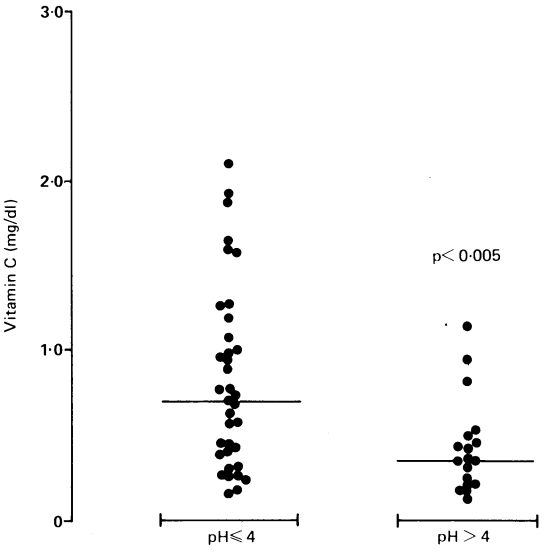

Fig. 2 *Plasma concentrations of vitamin C in hypochlorhydric patients compared with patients pH≤4. ——=median.*

When the relationship between intragastric and plasma concentrations was assessed, significant correlation ($p=0·002$) was achieved (Fig. 3).

VITAMIN C AND THE DIAGNOSTIC GROUPS

Individual values for intragastric concentrations of vitamin C in each of the diagnostic groups are shown in Figure 4. Patients in the normal endoscopy group had significantly higher concentrations compared with patients in the gastric cancer ($p<0·001$), pernicious anaemia ($p<0·005$), postoperative ($p<0·01$), gastric ulcer ($p<0·01$), and duodenal ulcer groups ($p<0·05$). Differences in intragastric concentrations between the latter five groups were not statistically significant.

Individual values for plasma concentrations of vitamin C in each of the diagnostic groups are shown in Figure 5. No significant differences in plasma vitamin C concentrations were detected between the groups with the exception of the postoperative group where values were significantly lower ($p<0·05$) than in the duodenal ulcer group.

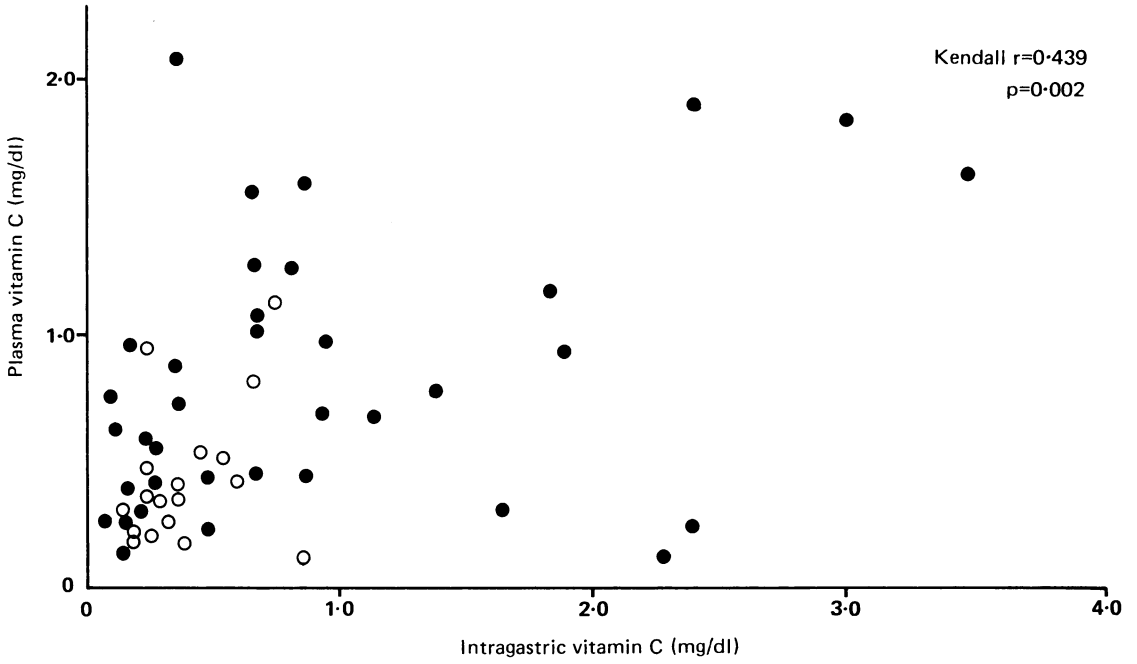

Fig. 3 *Correlation between intragastric and plasma concentrations of vitamin C.* ●=*pH*≤*4* ○=*pH*>*4.*

VITAMIN C AND GASTRIC MUCOSAL PATHOLOGY

Of the 52 patients who underwent gastric mucosal biopsy, histological evidence of chronic atrophic gastritis was found in 29 (56%). When intragastric concentrations of vitamin C were assessed in relation to mucosal pathology, the mean (SD) concentration in the group with atrophic gastritis was 0·59 (0·73) mg/dl compared with 0·72 (0·74) mg/dl in the group without the lesion ($0{\cdot}05<p<0{\cdot}1$). Plasma concentrations of vitamin C were similar in the two groups.

DAILY INTAKE OF VITAMIN C

Completed dietary assessment questionnaires were obtained from a total of 52 patients (71%) including all patients in the duodenal ulcer and gastric ulcer groups, nine of 13 in the normal endoscopy group (69%), three of four in the pernicious anaemia group (75%), and nine of 13 in the postoperative group (69%). Of the patients who completed dietary assessment questionnaires, 32 had had plasma vitamin C concentrations measured. Estimated daily intake of vitamin C (mean (SD)) was 56 (18) mg in the normal endoscopy patients, 47 (20) mg in the duodenal ulcer group, 57 (26) mg in the gastric ulcer group, 29 (10) mg in the pernicious anaemia patients and 48 (16) mg in the postoperative patients. Apart from uniformly low intake of vitamin C in the pernicious anaemia patients, the differences in estimated daily intake of vitamin C between the patient groups were not statistically significant. Estimated daily intake of vitamin C in patients with hypochlorhydria (n=15) was 49 (21) mg compared with 52 (25) mg in patients with a gastric pH≤4 (n=37) ($p>0{\cdot}05$). There was no significant correlation between estimated daily intake of vitamin C and intragastric or plasma concentrations (daily intake *v* intragastric concentrations, n=52, r=0·152, p=0·281; daily intake *v* plasma concentrations, n=32, r=0·084, p=0·650).

STABILITY OF VITAMIN C IN GASTRIC JUICE

Acidic gastric juice showed no significant change in basal or supplemented concentrations of vitamin C during incubation. By contrast in alkaline gastric juice basal concentration was reduced by one half and supplemented concentration by three quarters. There was no significant change in the pH of either the acidic or alkaline samples during incubation.

GASTRIC SECRETION OF VITAMIN C

Output of vitamin C increased significantly in all five subjects after pentagastrin injection, rising from a basal output (mean (SD)) of 0·42 (0·27) mg/hour to 1·65 (0·85) mg/hour at 30–40 min postpentagastrin ($p<0{\cdot}05$). Acid output increased from a basal level of 1·23 (1·30) mmol/hour to 16·42 (5·94) mmol/hour at 30–40 min postpentagastrin ($p<0{\cdot}001$) (Fig. 6).

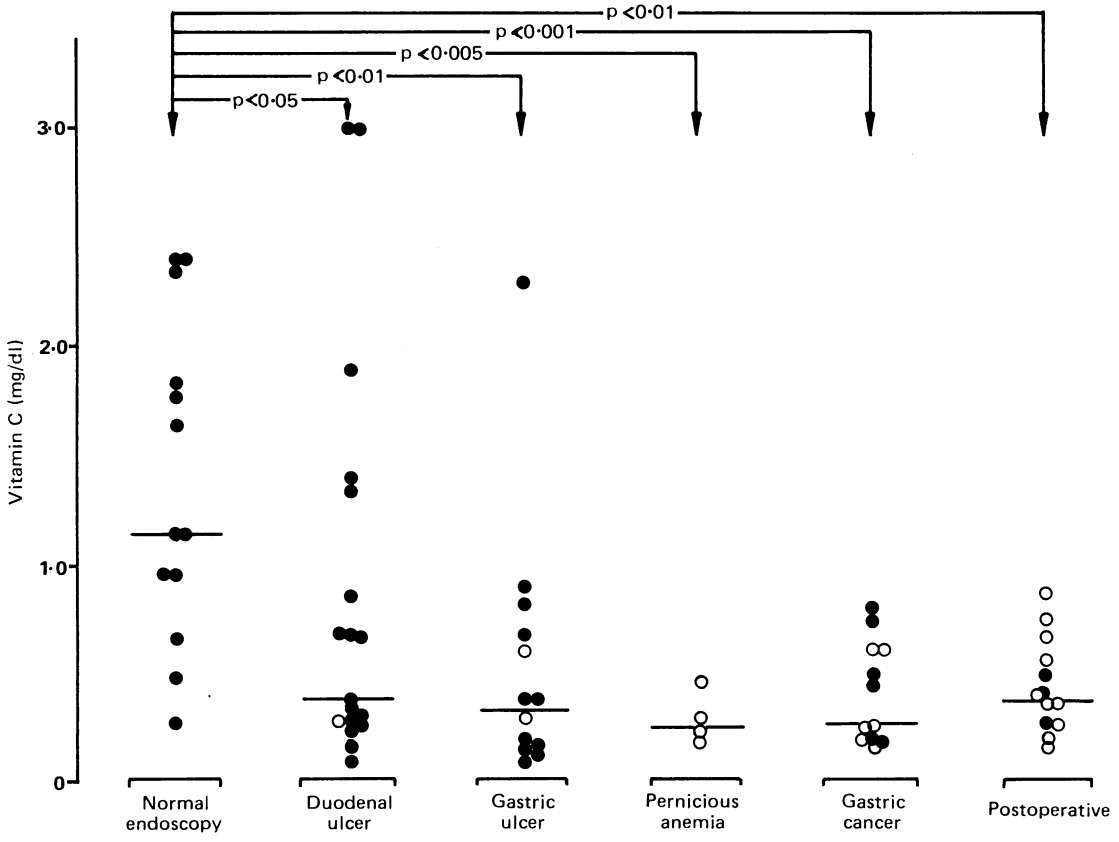

Fig. 4 *Intragastric concentrations of vitamin C and the patient groups.* ——*=median.* ●*=pH≤4 0=pH>4.*

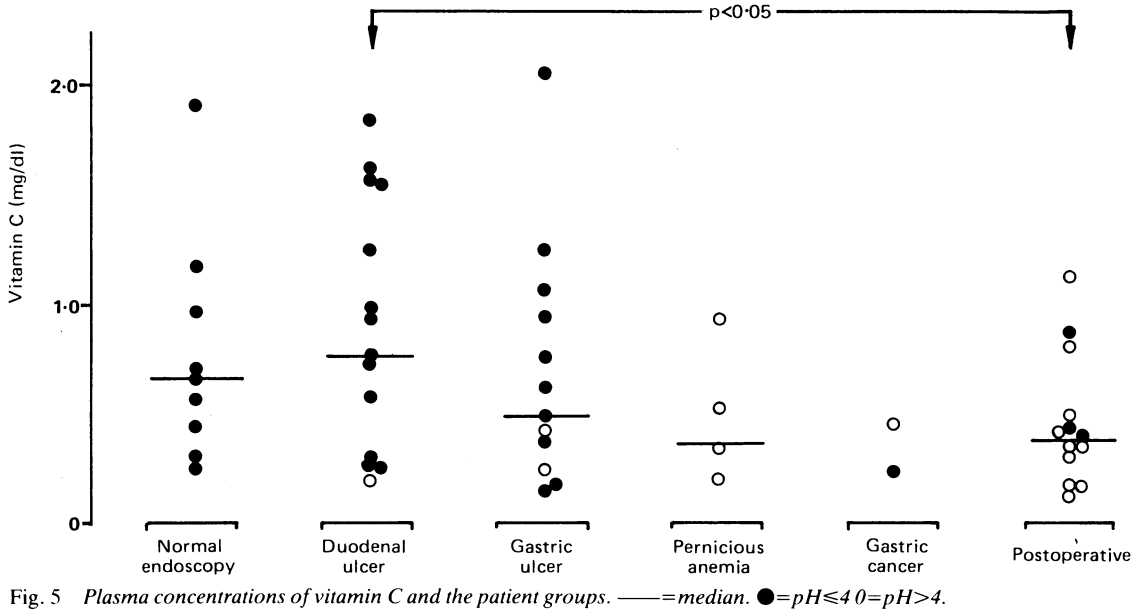

Fig. 5 *Plasma concentrations of vitamin C and the patient groups.* ——*=median.* ●*=pH≤4 0=pH>4.*

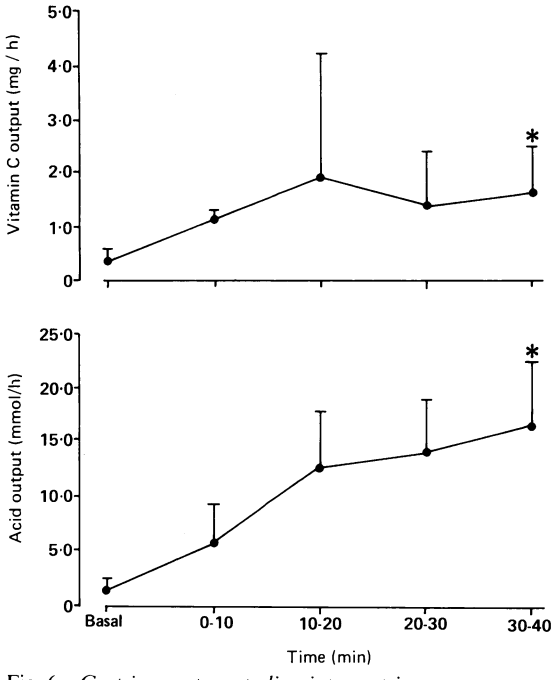

Fig. 6 *Gastric secretory studies: intragastric concentrations of vitamin C and pH after stimulation with pentagastrin. *●=mean (1 SD).*

Discussion

Significantly lower gastric juice concentrations of vitamin C were found in patients with hypochlorhydria compared with those having a pH≤4, although the estimated daily intake of vitamin C was similar in the two groups. There was a significant correlation between plasma and intragastric levels. *In vitro* studies on the stability of vitamin C showed a substantial fall in basal and supplemented concentrations over 24 hours in alkaline but not acidic gastric aspirate, suggesting that vitamin C may be chemically unstable under hypochlorhydric conditions. By allowing available nitrite to react 'unopposed' with appropriate amino substrates, low concentrations of vitamin C could conceivably permit the generation of N-nitroso derivatives in the stomach. This may be of particular importance in the hypochlorhydric stomach where high levels of nitrite are known to occur.[13]

Intragastric, but not plasma, concentrations of vitamin C were significantly higher in patients with normal endoscopic findings compared with those in all other diagnostic groups. Vitamin C concentrations were similar in patients with peptic ulcer, pernicious anaemia, gastric cancer, or after peptic ulcer surgery. In an earlier study, Freeman and Hafkesbring[14] also found lower vitamin C concentrations in gastric juice from patients with peptic ulceration, pernicious anaemia or gastric malignancy compared with normal control subjects. The finding of similar levels of vitamin C in duodenal ulcer patients, a group thought to be at low risk of gastric cancer,[15] and in patients with pernicious anaemia or after partial gastrectomy suggests that factors other than, or in addition to, vitamin C determine the increased risk of gastric cancer in these latter two conditions.[16,17]

When we assessed the relationship between vitamin C and gastric mucosal pathology, there was a strong trend towards lower intragastric concentrations in patients with atrophic gastritis. Rathbone *et al*[18] similarly found lower intragastric vitamin C concentrations in patients with gastritis. In animal studies performed over 30 years ago, Breidenbach and Kay[19] showed that chemically induced gastritis caused a significant fall in vitamin C concentrations in the stomach of the guinea pig and rat. Our gastric secretory studies, and those of Rathbone *et al*,[18] suggest that vitamin C is secreted by the stomach. It is conceivable, therefore, that secretory capacity for vitamin C may be impaired in the presence of gastritis, a condition usually present in patients with peptic ulceration.[20]

Further evidence that secretion of vitamin C may be a physiological property of gastric mucosal cells comes from vitamin C saturation studies. After saturation,[21] vitamin C concentrations in the blood and the amounts excreted in urine are in direct proportion to the intake suggesting simple quantitative balance whereas gastric juice concentrations rise far less proportionately suggesting a qualitative change in which cell function may be involved. In contrast with Hafkesbring *et al*[21] we found insignificant correlation between dietary intake of vitamin C and plasma concentrations but this may reflect the relatively small number of paired observations analysed, the wide variation in plasma levels of vitamin C and the known imprecision of dietary recall.

In conclusion, we have found low fasting concentrations of vitamin C in hypochlorhydric gastric juice where vitamin C may be chemically unstable. Patients with normal endoscopic findings have higher intragastric concentrations of vitamin C than patients with gastroduodenal disease. Vitamin C may be secreted in the human stomach. Finally, we speculate that variable concentrations of vitamin C may be a factor which influences endogenous nitrosation in the human stomach.

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